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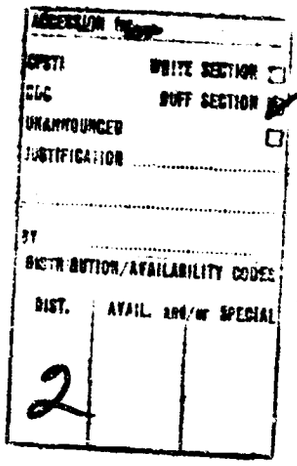
TECHNICAL MANUSCRIPT 577

ANTIBODY FOR CHLAMYDIA PSITTACI IN ASCITIC FLUIDS OF IMMUNIZED MICE IMPLANTED WITH SARCOMA 180

Altha C. Mumford

DECEMBER 1969

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland



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DEPARTMENT OF THE ARMY
Fort Detrick
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ANTIBODY FOR CHLAMYDIA PSITTACI IN ASCITIC FLUIDS OF IMMUNIZED MICE IMPLANTED WITH SARCOMA 180

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Medical Investigation Division
MEDICAL SCIENCES LABORATORIES

Project 1B662706A072

December 1969

In conducting the research described in this report, the investigator adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ACKNOWLEDGMENT

I am indebted to Dr. James T. Duff for guidance during these studies.

ABSTRACT

Ascitic fluids were collected from mice after immunization with nonviable vaccines for Chlamydia psittaci, challenge with the respective viable agent, and implantation of sarcoma 180. The fluids produced against the Borg, 6BC, and New Jersey strains contained useful titers of antibody demonstrable by neutralization and complement-fixation titrations. Neutralization was demonstrable against intraperitoneal challenge in mice, but was slight or negligible against intracerebral challenge in mice and against inoculation into the yolk sac of embryonated eggs.

I. INTRODUCTION*

Neutralizing antibodies to Chlamydia psittaci have been demonstrated in the sera of various laboratory animals.[1,2] However, routine preparation of suitable antisera is attended by practical difficulties, and high-titer, homotypic neutralizing antisera for the various strains of this group of agents have been available in very limited quantities. Methods in use for the production of immune ascitic fluids in mice[3-5] suggested a means for obtaining antisera for C. psittaci. The present paper describes application of this procedure for preparation of useful amounts of antibody to several strains of C. psittaci.

II. MATERIALS AND METHODS

A. SARCOMA CELLS

The strain of sarcoma 180 tumor cells used in these studies was maintained by routine implant passage in Swiss-Webster mice obtained from the Fort Detrick colony. Additional studies showed that sarcoma cells stored at -70 C for approximately 3 years produced abdominal distention in approximately 2 weeks, but after storage for 5 years, the time required to produce swelling was increased considerably.

B. VACCINES

Formalinized vaccines for the Borg, 6BC, New Jersey (NJ), and California Pigeon (CP) strains of C. psittaci were used.[6,**]

C. PRODUCTION OF ANTIBODY

Mice that survived vaccine antigenicity titrations were used. The mice had received three intraperitoneal (IP) injections of 0.5 ml of vaccine and had been challenged with 0.5 ml of a range of dilutions of yolk sac suspension of the pertinent viable agent. After about 2 weeks, survivors that had received the highest challenge doses (1,000 to 100,000 yolk sac LD_{50}) were implanted IP with approximately 2.5×10^6 sarcoma cells. Ascitic fluids were collected at 2- to 3-week intervals following implantations;

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the author to ascertain when and where it may appear in citable form.

** Dr. L.A. Page provided the NJ and CP strains.

20 to 30 ml of ascitic fluid were obtained from each mouse. Fluids from groups that had been challenged with the same strain were pooled, clarified by centrifugation at 10,000 rpm, inactivated at 56 C for 30 minutes, and stored at -20 C. Titers did not change appreciably over a period of 2 years or more.

D. NEUTRALIZATION TITERS

The tests were conducted by the varying virus - constant serum method, using infected yolk sac suspensions of the respective strains of C. psittaci. Equal amounts of the dilutions of agent and ascitic fluid were mixed, held at room temperature for 4 hours, then injected IP into 18- to 20-g mice. The mice were examined daily and deaths were recorded over a 14-day period. End points were determined according to Reed and Muench.[7] The log neutralizing capacity was found by subtracting the log of the mouse intraperitoneal LD_{50} ($MIPLD_{50}$) per ml of the immune ascitic fluid from that of the normal control ascitic fluid. Normal ascitic fluids had no apparent effect on the infectivity of yolk sac suspensions.

E. COMPLEMENT-FIXATION TITRATIONS

Complement-fixation tests with immune ascitic fluids were carried out using the Standardized Diagnostic Complement Fixation Method and Adaptation to Micro Test.[8] Cell wall preparations of each strain were used as antigens.[9] The dilution of ascitic fluid showing 30% hemolysis was taken as the end point. Negative results were obtained with normal ascitic fluids; anticomplementary activity of the fluids was low.

III. RESULTS

A. ANTIBODY IN ASCITIC FLUIDS

Neutralization titrations on four Borg immune ascitic fluid drainages obtained over a period of 39 days and experiments on the effect of dilution on the neutralizing activity are shown in Table 1. Ascitic fluids obtained from mice that had not been immunized or challenged were used as controls. Ascitic fluids obtained during the first three drainages from the immune group of mice exhibited a high neutralizing capacity. Greater than 10^6 $MIPLD_{50}$ was neutralized with day 16 fluid, and greater than 10^5 $MIPLD_{50}$ was neutralized by fluid obtained on days 22 and 29. By the fourth drainage on day 39, the antibody level was considerably lower. Immune ascitic fluid diluted 1:32 neutralized approximately 10 $MIPLD_{50}$ of the Borg agent. Ascitic fluid from mice immunized with 6BC, NJ, and CF vaccines and challenged with the homologous strains neutralized 2.4, >5.8, and >0.4 log $MIPLD_{50}$/ml respectively (Table 2). Ascitic fluid from mice that had been immunized with vaccine but not challenged did not have neutralizing capacity.

B. PASSIVE IMMUNITY

The duration of passive protection obtained in mice by IP injection of 0.5 ml of undiluted Borg immune ascitic fluid is shown in Table 3. Groups of animals were challenged IP with various dilutions of infected yolk sac material of the Borg agent 1, 5, and 15 days following immunization. Passive protection was strongly evident on the 1st and 5th days following administration of ascitic fluid. No end point was obtained in the 15-day challenge, but it is evident that immunity had declined between 5 and 15 days.

C. SPECIFICITY OF NEUTRALIZATION AND COMPLEMENT FIXATION

The extent of cross reaction of the Borg and 6BC immune ascitic fluids was investigated (Table 2). Borg immune fluid did not neutralize 6BC agent, and 6BC immune fluid neutralized less than 10 $MIPLD_{50}$ of Borg agent. The neutralizing capacity of the 6BC preparations was less than that of the Borg fluids. Complement-fixation titers of the various ascitic fluids are also summarized in Table 2.

D. EFFECT OF HOST OR ROUTE

No neutralization was obtained in embryonated eggs inoculated by the yolk sac route, and only a small amount of neutralization was obtained in mice inoculated by the intracerebral (IC) route.

TABLE 1. PERSISTENCE OF ANTIBODY IN BORG IMMUNE ASCITIC FLUID AND FINAL DILUTION END POINTS

Type Ascitic Fluid[a]	Days after Implantation	Final Dilution of Ascitic Fluid	Log $MIPLD_{50}$/ml	Log Neutralizing Capacity
Immune - drainage 1	16	1:2	<1.3	>6.2
Normal control		1:2	7.5	
Immune - drainage 2	22	1:2	<1.3	>6.4
		1:8	4.3	2.4
		1:32	6.6	1.1
Normal control		1:2 1:8	7.7	
Immune - drainage 3	29	1:2	<1.3	>4.9
Immune - drainage 4	39	1:2	>5.3	<0.9
Normal control		1:2	6.2	

a. Ascitic fluids from four to eight mice were pooled.

TABLE 2. COMPLEMENT-FIXATION AND NEUTRALIZATION TITERS OF ASCITIC FLUIDS

Type Ascitic Fluid	Test Strain	CF Titer (Cell Wall Antigen)	Log $MIPLD_{50}$/ml		Log Neutralizing Capacity
			Immune Fluid	Normal Fluid	
Borg	Borg	256	<3.3	7.1	>3.8
	6BC	4	7.1	5.7	-1.4
6BC	6BC	16	3.3	5.7	2.4
	Borg	8	6.3	7.1	0.8
NJ	NJ	64	2.5	>8.3	>5.8
CP	CP	4	<1.3	1.7	>0.4

TABLE 3. PERSISTENCE OF PASSIVE IMMUNITY PRODUCED BY BORG IMMUNE ASCITIC FLUID

Interval between Immunization and Challenge, days	Group	Mice Surviving/Total Inoculated — Log of Dilution of Borg Agent						
		-1	-2	-3	-4	-5	-6	-7
1	Immune	1/3		3/3		3/3		3/3
	Control			0/3		1/3		3/3
5	Immune	2/3		3/3		3/3		
	Control			0/2		1/3		3/3
15	Immune	1/2	0/3	0/3	1/3			
	Control				0/2	0/3	0/3	2/3

IV. DISCUSSION

Previous investigators had found that sera of immunized rabbits, mice, guinea pigs, and monkeys demonstrated little or no neutralizing capacity.[10] Hilleman,[11] using a system of infectivity scores based on extent of lung consolidation in mice, recorded a high neutralizing capacity in the serum of roosters immunized with mouse pneumonitis agent; no protection was obtained with the serum of similarly immunized rabbits. Although ascitic fluid titers were not as high as those obtained with convalescent rooster serum, preparation of ascitic fluids is more convenient and less hazardous than collection of serum from infected avian hosts. The ascitic fluids appear useful for studies on the antigenic and immunogenic structure of this group of microorganisms, and may prove applicable to other chlamydial agents. The ascitic fluids did not neutralize the agents in eggs or protect mice inoculated IC. This indicates that host susceptibility and route of inoculation are important considerations in determining neutralizing capacity.

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Unclassified
Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
Department of the Army
Fort Detrick, Frederick, Maryland, 21701

2a. REPORT SECURITY CLASSIFICATION
Unclassified

2b. GROUP

3. REPORT TITLE
ANTIBODY FOR CHLAMYDIA PSITTACI IN ASCITIC FLUIDS OF IMMUNIZED MICE IMPLANTED WITH SARCOMA 180

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (First name, middle initial, last name)
Altha C. Mumford

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
December 1969	13	11

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
b. PROJECT NO. 1B622706A072	Technical Manuscript 577
c. Task-Work Unit 03-001	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
d. DD 1498 Agency Accession DA OL 0264	CMs 6640 SMUFD-AE-T 49567

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11. SUPPLEMENTARY NOTES	12. SPONSORING MILITARY ACTIVITY
	Department of the Army Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Ascitic fluids were collected from mice after immunization with nonviable vaccines for Chlamydia psittaci, challenge with the respective viable agent, and implantation of sarcoma 180. The fluids produced against the Borg, 6BC, and New Jersey strains contained useful titers of antibody demonstrable by neutralization and complement-fixation titrations. Neutralization was demonstrable against intraperitoneal challenge in mice, but was slight or negligible against intracerebral challenge in mice and against inoculation into the yolk sac of embryonated eggs.

14. Key Words

Chlamydia psittaci
Ascitic fluid
Sarcoma 180
Antibody
Neutralization

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